

Identification of *Wolbachia pipientis* and phylogenetic studies of *plodia interpunctella* populations in different cities of Pakistan

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Wolbachia pipientis is genus of gram-negative bacteria which infect the members of phylum arthropoda, mainly affects the class insecta and some nematodes. *Wolbachia pipientis* are the common bacterial symbionts of the insects. About 25 to 70% of insect species are estimated to be the host of *Wolbachia pipientis*. Infection of these bacteria can alter the reproduction and biology of the host. Even some host species can not reproduce without *Wolbachia pipientis* infection. Due to this reason, *Wolbachia pipientis* are used to control the population of pest species. A major research objective is the detection of *Wolbachia pipientis* in the *Plodia interpunctella* populations of different cities of Pakistan leading towards its phylogenetic descriptions through molecular markers. *Plodia interpunctella* commonly called Indian meal moth. This insect is the major and most destructive pest of food grains such as, rice, maize and other cereals. The presence of this pest is relating with occurrence of such grains in homes and grocery stores. Indian meal moth feeding reduces the dry weight of stored grains. This study was done to estimate the economic and yield losses of food grains due to *Plodia interpunctella* in the different cities of Pakistan such as (Faisalabad, Jhang and Layyah) from Punjab, (Peshawar) from KPK and (Hyderabad) from Sindh. Firstly, the Collection of *Plodia interpunctella* was completed from mentioned areas, and the pest was identified through morphologically, molecular analysis, eggs and larval instar. The results of our study showed that, the pest infection rate was not similar in all the cities. Its major attack was identified in Faisalabad, Jhang, Layyah, Peshawar and Hyderabad gradually. During the study, pest infection rate was calculated in four months, July, August, September and October. The attack of pest was maximum in the start of July and minimum at the end of October in stored grains. The results were calculated by using DNA extraction. The statistical analysis was performed through SPSS.

Keywords: Indian meal Moth, arthropoda, *Wolbachia pipientis*, destructive pest, molecular analysis, stored grains.

INTRODUCTION

The largest animal group which includes more than 75% of all known animal species is known as Arthropodes, and the first living form to emerge on Earth are insects, which are members of the phylum Arthropoda (Kim *et al.*, 2014; Shepard *et al.*, 2014 and Silhacek *et al.*, 1972). The majority of domesticated animals as well as humans eat cereal grains. *Plodia interpunctella* are harmful stored-grain pests that are widely dispersed over the world and are members of the order "Lepidoptera." The Pyralidae family includes the genus *Plodia* *interpunctella* (Triggs *et al.*, 2012). The most significant pest

of products usually found in homes or supermarkets that are stored is said to be the stored grains pest *plodia interpunctella* (Johnson *et al.*, 1992 and Cowan *et al.*, 2009).

Adult meal moths are around 3/8-inch (10 mm) length at repose, with a wing spread of about 5/8 inch (14mm). They have bronze-colored wings. The forewings' lower half is a rusty red-brown tone, while the front half has a grayish-white hue (Lewis *et al.*, 2011; Kuwahara *et al.*, 1971 and Tang *et al.*, 2008)

Plodia interpunctella laid her eggs on the food sources for larvae, such as dried fruits, cereals, grains that had been stored, and spices. The Indian meal moth's eggs have a

Raza, E.B., S.A. Khan, M.S. Anees, M. Kamal, S. Masud, A. Hamza, M. Tahir, M. Asghar³, G.Z. Khan and M.S. Nawaz. 2023. Identification of *wolbachia pipientis* and phylogenetic studies of *plodia interpunctella* populations in different cities of Pakistan. Journal of Global Innovations in Agricultural Sciences 11: 217-227.

[Received 23 Mar 2023; Accepted 17 Jun 2023; Published 27 Jun 2023]



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greyish white appearance (Bryson *et al.*, 2014). Eggs are typically placed directly on the larval food source, either singly or in clusters. Eggs can hatch in between 7-8 days at 20 °C and 3-4 days at 30 °C (Bourtzis *et al.*, 2008).

Their hue is often off-white, but depending on the food source, it has been seen to be pink, brown, or almost green. About half an inch (1.27 cm) long, the mature larvae. Larvae can develop to maturity in 6-8 weeks at temperatures ranging from 18 to 35 °C (Zhu *et al.*, 2005; Baldo *et al.*, 2006 and Werren *et al.*, 2008).

Either without protection or in a silken cocoon, the larvae pupate. The pupae have a light brown colour and range in length from 1/4 to 2/5 of an inch (6 to 11 mm) (Zhao, 2013). At 20 °C and 30 °C, the pupal stage might last anywhere between 15 and 30 days. Adults have a wing span of about 5/8 inch and measure about 1/2 inch (12.7 mm) in length (16 to 20 mm) (Ivanov *et al.*, 2011).

It takes between 27 and 305 days to complete the life cycle. After mating, the *Plodia interpunctella* female can produce between 30 and 400 eggs. The time it takes for eggs to hatch spans from 7 to 30 days, larvae live for 6 to 8 weeks, and pupae can live for 15 to 20 days (Karami *et al.*, 2016; Gottlieb *et al.*, 2006 and Ros v, 2012)

Endosymbiotic Bacteria *Wolbachia pipientis*

A potential endo-symbiotic microbial biocontrol agent is the gram-negative bacteria *Wolbachia pipientis*, which is inherited from the mother (Wijayaratne *et al.*, 2012). It's a Rickettsia possession (Narita *et al.*, 2009 and Hrudová *et al.*, 2017). It has been discovered using electron microscope, bacteria have three body layers: one external and two internal body layers. There is a vacuole in the outermost layer. *Wolbachia pipientis* was given its name since it was initially discovered in the ovaries of *Culex pipientis* in 1924 (Gutzwiller *et al.*, 2015; Mullen *et al.*, 2001 and Vukajlovic *et al.*, 2012).

MultiLocus sequence typing is used to classify bacterial strains (MLST). Since the most effective applications are produced for the examination of harmful bacteria, Maiden and colleagues first developed this technology as a means for strain racial of meningococcus (Tagami *et al.*, 2004; Bowditch *et al.*, 1996 and Kageyama *et al.*, 2002). With the help of this approach, strains can now be typed and sorted, and a database that details *Wolbachia* ecology, diversity, and evolution is now available (Lewis *et al.*, 2021 and Kyei-Poku *et al.*, 2005).

According to (Baldo *et al.*, 2005) the endosymbiont *Wolbachia* uses a variety of phenotypic mechanisms, including parthenogenesis, feminization, cytoplasmic incompatibility, and the destruction of male sperm, to manipulate and alter the host reproductive systems (Yildirim *et al.*, 2013 and Dyson *et al.*, 2002).

Parasitic role of *Wolbachia pipientis*: In order to influence the reproductive systems of a variety of nematodes and arthropods, *wolbachia*, one of the most aggressive maternally

inherited endosymbiotic proteobacteria, inhabits these organisms. Intercellular bacterial parasitism of invertebrates is quite frequent and is essential for controlling host biological processes as development, gene expression, and immune systems (Baldo *et al.*, 2006 and Serbus *et al.*, 2008).

Objective of study:

- To identified the population of *P. interpunctella* in different districts of Pakistan.
- Investigate the infection of *Wolbachiapipientis* in *P. interpunctella* populations.
- To explore the diversity of *W.pipientis*.
- Determine the effects of *W.pipientis* on the mitochondrial genome of their host.
- The phylogenetic study of *P. interpunctella*.

MATERIALS AND METHODS

In a recent study, the common endosymbiont bacterium *Wolbachia pipientis* was found in Pakistani *plodia interpunctella* populations and its phylogenies.

Study Area: The several Pakistani districts were chosen for the *Plodia interpunctella* collection.

District Faisalabad: In the Punjab, the city of Faisalabad is in the Centre. *Plodia interpunctella* populations were chosen from two Faisalabad locations (Rafhan Maize Products Co.Ltd. and Rehman Flour Mills).

District Jhang: Jhang is situated on the Chenab River's. The Farooq and Zafar Flour Mills in Jhang were the two locations where the *plodia* populations were gathered.

District Layyah: Layyah district is located in the southern part of province, Punjab. The samples were collected from Muhamad Ishaq Cheema Flour Mill Kachehry and Asad Flour Mill Fateh Pur Road.

District Peshawar: Peshawar is the capital of KPK, near to Khyber Pass. The climate is local steppe type. One location (Rehmat Flour and General Mills near Charsadda Road) was selected for Indian meal moth collection.

District Hyderabad: Hyderabad is the 2nd largest city of Sindh. In Hyderabad, major grain crops that are cultivated are rice and wheat. One location (Rafhan Maize Products Pvt.Ltd.) was selected for *Plodia interpunctella* collection.

Sample Collection: Placing traps in grain mills that were being stored allowed for the collection of *Plodia interpunctella* samples. Sticky yellow bands are the most widely used trap for collecting *Plodia interpunctella* from adults (Silhacek *et al.*, 2003 and Sunish *et al.*, 2011).

Identification of *Plodia interpunctella* Species: The *Plodia interpunctella* population was gathered from five distinct Pakistani districts. Under a microscope, their morphological characteristics served as the basis for their identification. Nevertheless, molecular/DNA bar coding techniques were used to further identify and categorise these obtained samples (Li *et al.*, 2015 and Sambaraju *et al.*, 2008).



Rearing of *Plodia interpunctella* on grains of Host Crops: *Plodia interpunctella* populations that had been collected were produced in a lab using the grains of particular host plants. Seeds of wheat, maize, gramme, and sorghum served as the hosts for raising. *Plodia interpunctella* reproduction on grain storage (Ming *et al.*, 2015; Pattabhiramaiah *et al.*, 2012 and Sungpradit *et al.*, 2010).

DNA Extraction: DNA was extracted manually and using extraction kits from the samples that were collected. Three techniques were employed manually: CTAB, TNE buffer, and phenol techniques. The Gene Mark Company's extraction kit was employed, and the procedure for extracting DNA was adhered to as specified by the product's manufacturer (Tagami *et al.*, 2004).

Manual Extraction Methods ctab buffer method: For the CTAB buffer method, following procedure was performed. First, 400 µl of CTAB standardising buffer and a clean pestle were used to homogenise larvae, pupae, and adults in order to extract their DNA (Fujii *et al.*, 2001). Each sample received 100 µl of Proteinase-K at a concentration of 20 µg/l. Then samples were vortexed and incubated for two to three hours at 65°C. A supernatant layer formed at the top of the tube, which was then transferred into a new tube and mixed with 400–500 µl of chloroform–isoamyl alcohol. The mixture was once more vortexed and centrifuged. Afterwards, a new eppendorf tube was used to transfer the upper aqueous layer. 300 to 400 µl of 100% ethanol were used to precipitate the DNA. 30 to 50 µl of sterile water were used to resuspend the DNA pellet (d3H2O) (Graham *et al.*, 2012 and Kawasaki *et al.*, 2016).

TNE Buffer Method: For the TNE buffer method, following procedure was performed. Initially, marked Eppendorf tubes were used to collect the samples. With a clean mortar and pestle, 300 µl of extraction buffer was mixed with the mixture. Proteinase K was dissolved in 10 µl of water and carefully vortexed. The mixture was incubated for 1–2. 650 ml of chloroform-isoamyl alcohol and 250 ml of 4.5 M NaCl were then added, and the mixture was centrifuged. After centrifugation, the DNA pellet was dried and diluted in 30–50 µl of sterile ddH2O after being washed with 100 µl of 70% ethanol (Werren *et al.*, 2008; Ilinsky *et al.*, 2017).

Phenol Method: For the Phenol method, following procedure was performed. Firstly, Consume 5–10 mg of tissue or insect with eppendorf. Pour 350 µl of the tris buffer solution. Each sample should receive 20 µl of proteinase K (20 mg/ml). Incubate the samples over night at 56°C. Add 400 µl of phenol and centrifuge for 10 minutes at 13,000 rpm. Add 1/10 of the solution's sodium acetate. 500 µl of 100% cold iso-propanol should be added, and the mixture should be placed in the freezer for the night. Once more, centrifuge the remaining ethanol in the supernatant for five minutes at 8000 rpm. The DNA pellet is air dried. By adding 50 µl of PCR water or TE buffer, dissolve the DNA (Kolasa *et al.*, 2017; Sasaki *et al.*, 1999).

DNA Extraction Through Kits: For the method, following procedure was performed. The tissue or insect should first be chopped placed microcentrifuge tube. 1.5 ml of a microcentrifuge tube should contain 600 µl of the extraction solution. Add 20 µl of proteinase K solution and vortex it in the microcentrifuge tube. The samples should be incubated for 0.5 to 3 hours, or more, at 56 °C in a water bath or incubator, with periodic vortexes of 5 to 10 seconds (Ahmed *et al.*, 2016). Each sample received 4 µl of RNase A, which was added, and was incubated at 37 °C for 15–30 minutes. Each sample should get 200 µl of the protein precipitation solution, which you should vortex well. Centrifuge the samples at room temperature for 3 minutes at high speed (14,000 rpm). Next, centrifuge once more after 5 minutes of incubation on ice. Put the supernatant in a fresh Eppendorf tube without disturbing the pellet. 600 µl of isopropanol should be added, and it should be properly mixed by repeatedly spinning the tube until the DNA firmly precipitate forms (Chen, 2012; Kikuchi, 2003).

DNA Quantification: Nano drop and UV Spectrophotometers were used to quantify DNA.

DNA Quantification through UV Spectrophotometer: The value 00.00 was built up using tap water as the control.

- Following the computation to get the DNA concentration, absorbance at 260 and 280 nm wavelengths of UV light were used to determine the OD value of each sample.
- To evaluate the DNA's quality Each isolated DNA sample was processed through gel electrophoresis five times.

DNA Quantification: Put 2 µl of PCR water or sterile ddH2O to the Nano Drop lens and set the value to 00.00 as the control.

- Next, place 1 µl of each sample on the lens and note the absorbance at 260 and 280 nm as well as the amount of DNA (g/µl).
- Five litres of each isolated DNA sample were processed through gel electrophoresis to test the DNA's purity.

RESULTS

In order to better understand the naturally occurring endosymbiont *Wolbachia pipientis* in *Plodia interpunctella* populations in the several towns of Pakistan, a genetic analysis was carried out.

***Plodia interpunctella* population in five districts of Pakistan:** The five districts of Pakistan were sampled for *Plodia interpunctella*, and the highest population densities were found in Faisalabad, Jhang, Layyah Hyderabad, and Peshawar, respectively.

***Plodia interpunctella* population during four months:** Because there were enough stored grains of host crops including maize, wheat, gramme, and sorghum available between the months of August and September up until the



month of October, the maximum population of *Plodia interpunctella* was seen during those months.

Month wise population of *Plodia interpunctella* in Faisalabad district: Data was gathered week by week over the course of four months. The population of *Plodia interpunctella* showed growing trends throughout the first three weeks of every month. The third and fourth weeks of September saw the greatest population collection. Population trends that decreased throughout October were evident.

Month wise population of *Plodia interpunctella* in Jhang district: Data was gathered week by week over the course of four months. Rising trends in the population of *Plodia interpunctella* were seen in the first and second weeks of July. The third and fourth weeks of August were when the largest population was counted. Population trends that decreased throughout October were evident.

Month wise population of *Plodia interpunctella* in Layyah district: Data was gathered week by week over the course of four months. *Plodia interpunctella* had a large population during the first three weeks of July. Within the months' samples and between the months' collections, a little amount of variation was discovered. Up until the third week of August, the first and second weeks of September saw the highest population. A relatively small population of *Plodia interpunctella* was gathered in the months of July and October.

Month wise population of *Plodia interpunctella* in Hyderabad district: The Hyderabad district showed a comparatively high *plodia interpunctella* population rate. *Plodia* population was counted over the course of the four-month survey, and it was discovered that it peaked from July to the first week of October. The final weeks of October revealed slightly declining trends in population.

Month wise population of *Plodia interpunctella* in Peshawar district: The first two weeks of July saw the highest population trend. Yet, compared to the population of other districts, Peshawar's samples are fewer in number. The population had varying patterns in its size, and in the month of October, those trends began to wane.

***Plodia interpunctella* population on different host grains:** The populations of Indian meal moth were reared on their different host grains e.g., wheat, maize, sorghum and grams. They attack on different stored grains like wheat, maize, sorghum and grains. They showed higher preference for wheat, maize, sorghum and grains respectively.

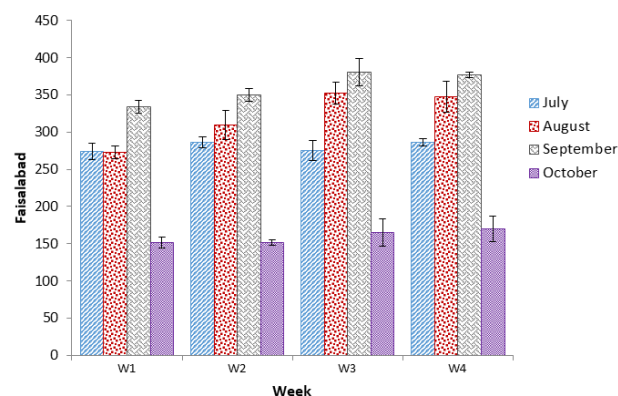


Figure 1. Population of Indian meal moth in Faisalabad during four months

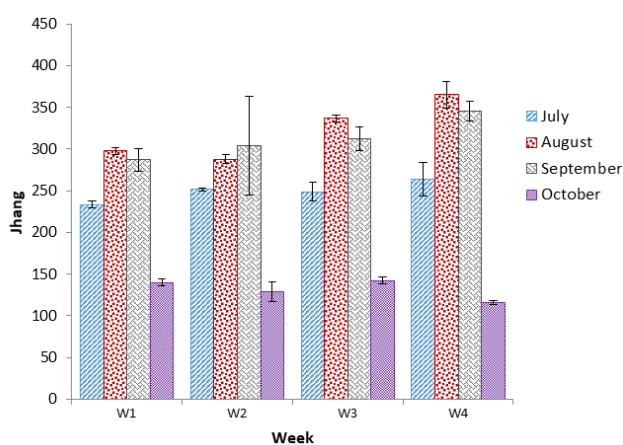


Figure 2. Population of Indian meal moth in Jhang during four months

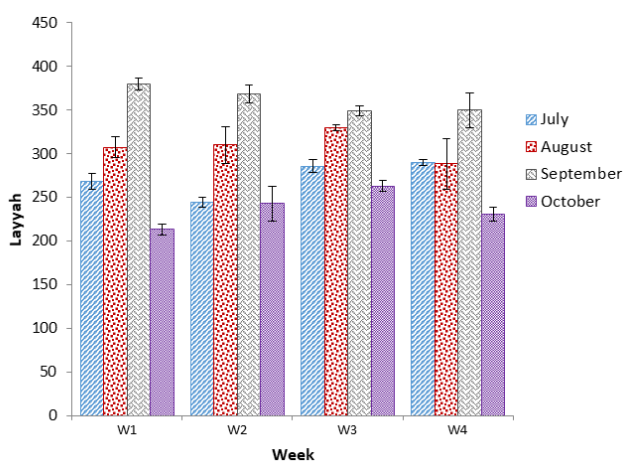


Figure 3. Population of Indian meal moth in Layyah during four months



Table 1. Analysis of variance table for district Faisalabad

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Month	3	271367	90456	180.78**
Week	3	11091	3697	7.39**
Month x Week	9	7024	780	1.56 ^{NS}
Error	32	16012	500	
Total	47	305495		

NS = Non-significant (P<0.05); ** = Highly significant (P<0.01)

Month x Week interaction means

Week	Month				Mean
	July	August	September	October	
W1	273.67±10.81	272.67±8.09	334.00±8.02	151.33±7.31	257.92±20.35B
W2	286.33±7.54	309.67±19.88	350.33±8.45	152.00±3.61	274.58±22.97AB
W3	275.33±13.20	352.67±14.68	380.67±17.95	165.00±18.93	293.42±26.15A
W4	286.33±5.24	347.33±20.38	376.67±3.33	170.00±17.56	295.08±24.60A
Mean	280.42±4.50C	320.58±12.01B	360.42±7.40A	159.58±6.27D	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Table 2. Analysis of variance table for district Jhang

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Month	3	275407	91802	97.48**
Week	3	8476	2825	3.00*
Month x Week	9	11077	1231	1.31 ^{NS}
Error	32	30137	942	
Total	47	325097		

* = Significant (P<0.05); ** = Highly significant (P<0.01)

Month x Week interaction means

Week	Month				Mean
	July	August	September	October	
W1	233.33±4.41	297.67±3.84	287.00±13.28	140.00±4.04	239.50±19.09B
W2	251.67±2.03	288.00±5.29	304.33±58.97	129.33±11.92	243.33±24.35B
W3	249.00±10.69	336.67±3.84	312.67±14.44	142.00±4.04	260.08±23.06A
W4	264.00±19.86	365.33±15.76	345.00±11.85	116.00±2.65	272.58±30.16A
Mean	249.50±5.92B	321.92±10.06A	312.25±14.91A	131.83±4.24C	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Table 3. Analysis of variance table for district Layyah

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Month	3	102223.8	34074.6	66.09**
Week	3	2282.8	760.9	1.48 ^{NS}
Month x Week	9	10172.7	1130.3	2.19*
Error	32	16498.7	515.6	
Total	47	131178.0		

* = Significant (P<0.05); ** = Highly significant (P<0.01)

Month x Week interaction means

Week	Month				Mean
	July	August	September	October	
W1	268.33±8.99d-g	307.33±11.78b-e	380.00±6.81a	213.00±6.08g	292.17±18.70A
W2	244.33±6.01efg	310.00±20.82b-e	368.67±10.37ab	242.67±19.92efg	291.42±17.09A
W3	285.67±7.13c-f	330.00±3.21a-d	348.67±5.78abc	263.00±6.43d-g	306.83±10.58A
W4	289.33±3.48c-f	288.33±29.28c-f	350.00±20.01abc	230.67±8.21fg	289.58±14.92A
Mean	271.92±6.08C	308.92±9.23B	361.83±6.51A	237.33±7.40D	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).



Table 4. Analysis of variance table for district Hyderabad

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Month	3	21176.8	7058.9	19.25**
Week	3	4511.8	1503.9	4.10*
Month x Week	9	10714.1	1190.5	3.25**
Error	32	11731.3	366.6	
Total	47	48133.9		

* = Significant (P<0.05); ** = Highly significant (P<0.01)

Month x Week interaction means

Week	Month				Mean
	July	August	September	October	
W1	312.00±2.89a-d	348.67±7.88ab	343.67±11.84abc	337.00±9.85a-d	335.33±5.66A
W2	333.00±5.20a-d	355.33±2.91a	314.00±14.73a-d	290.33±2.60cd	323.17±7.99AB
W3	308.33±18.11a-d	363.33±15.77a	365.00±17.39a	283.00±4.16d	329.92±12.44AB
W4	279.33±13.25d	344.33±10.68abc	321.00±13.86a-d	293.00±3.21bcd	309.42±8.93B
Mean	308.17±7.60B	352.92±4.94A	335.92±8.68A	300.83±6.84B	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Table 5. Analysis of variance table for district Peshawar

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Month	3	27246.8	9082.3	26.54**
Week	3	27174.9	9058.3	26.47**
Month x Week	9	26131.6	2903.5	8.48**
Error	32	10952.7	342.3	
Total	47	91505.9		

** = Highly significant (P<0.01)

Month x Week interaction means

Week	Month				Mean
	July	August	September	October	
W1	223.00±03.06a	143.00±06.24c-f	163.00±05.51b-e	126.33±1.76d-g	163.83±11.19A
W2	207.33±01.45ab	169.33±07.51a-d	194.33±03.53abc	111.67±0.67efg	170.67±11.21A
W3	109.67±04.81efg	175.67±06.89a-d	128.33±24.50d-g	101.67±5.55fg	128.83±10.34B
W4	123.33±15.94d-g	159.33±23.71b-e	88.67±10.93fg	82.67±5.90g	113.50±11.39B
Mean	165.83±15.47A	161.83±6.77AB	143.58±13.25B-E	105.58±5.10C	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Table 6. Analysis of variance table for population of all districts

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
District (D)	4	923241	230810	432.78**
Month (M)	3	496981	165660	310.62**
Week (W)	3	2099	700	1.31 ^{NS}
D x M	12	200442	16703	31.32**
D x W	12	51438	4286	8.04**
M x W	9	17414	1935	3.63**
D x M x W	36	47705	1325	2.48**
Error	160	85332	533	
Total	239	1824651		

* = Significant (P<0.05); ** = Highly significant (P<0.01)



Month x Week interaction means

Week	Month				Mean
	July	August	September	October	
W1	262.07±8.85cd	273.87±18.92bcd	301.53±20.46ab	193.53±20.89e	257.75±10.17A
W2	264.53±11.50cd	286.47±17.48abc	306.33±19.35a	185.20±18.94e	260.63±10.26A
W3	245.60±19.38d	311.67±18.85a	307.07±25.47a	190.93±19.14e	263.82±12.05A
W4	248.47±17.60d	300.93±21.64ab	296.27±28.58ab	178.47±20.65e	256.03±12.69A
Mean	255.17±7.37B	293.23±9.58A	302.80±11.59A	187.03±9.73C	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Month x District interaction means

Week	Month				Mean
	July	August	September	October	
Faisalabad	280.42±4.50efg	320.58±12.01bcd	360.42±7.40a	159.58±6.27ij	280.25±11.64C
Jhang	249.50±5.92gh	321.92±10.06bcd	312.25±14.91cde	131.83±4.24jk	253.88±12.00D
Layyah	271.92±6.08fg	308.92±9.23cde	361.83±6.51a	237.33±7.40h	295.00±7.63B
Peshawar	165.83±15.47i	161.83±6.77ij	143.58±13.25ij	105.58±5.10k	144.21±6.37E
Hyderabad	308.17±7.60cde	352.92±4.94ab	335.92±8.68abc	300.83±6.84def	324.46±4.62A

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Month x District x Week interaction means

District	Week	Months				Mean
		July	August	September	October	
Faisalabad	W1	273.67±10.81g-q	272.67±8.09h-r	334.00±8.02a-j	151.33±7.31y-e	257.92±20.35GHI
	W2	286.33±7.54d-q	309.67±19.88a-n	350.33±8.45a-h	152.00±3.61x-e	274.58±22.97D-H
	W3	275.33±13.20g-q	352.67±14.68a-g	380.67±17.95a	165.00±18.93v-d	293.42±26.15B-F
	W4	286.33±5.24d-q	347.33±20.38a-i	376.67±3.33ab	170.00±17.56t-c	295.08±24.60B-E
Jhang	W1	233.33±4.41m-w	297.67±3.84b-o	287.00±13.28d-p	140.00±4.04z-e	239.50±19.09I
	W2	251.67±2.03k-s	288.00±5.29d-p	304.33±58.97a-n	129.33±11.92a-e	243.33±24.35HI
	W3	249.00±10.69l-t	336.67±3.84a-j	312.67±14.44a-l	142.00±4.04z-e	260.08±23.06F-I
	W4	264.00±19.86j-r	365.33±15.76a-d	345.00±11.85a-i	116.00±2.65b-e	272.58±30.16E-I
Layyah	W1	268.33±8.99i-r	307.33±11.78a-n	380.00±6.81a	213.00±6.08p-z	292.17±18.70B-F
	W2	244.33±6.01l-u	310.00±20.82a-m	368.67±10.37abc	242.67±19.92l-v	291.42±17.09B-F
	W3	285.67±7.13e-q	330.00±3.21a-k	348.67±5.78a-h	263.00±6.43j-r	306.83±10.58A-D
	W4	289.33±3.48d-p	288.33±29.28d-p	350.00±20.01a-h	230.67±8.21n-x	289.58±14.92C-G
Peshawar	W1	223.00±3.06o-y	143.00±6.24z-e	163.00±5.51w-d	126.33±1.76b-e	163.83±11.19J
	W2	207.33±1.45q-a	169.33±7.51u-c	194.33±3.53r-b	111.67±0.67cde	170.67±11.21J
	W3	109.67±4.81cde	175.67±6.89s-c	128.33±24.50a-e	101.67±5.55cde	128.83±10.34K
	W4	123.33±15.94b-e	159.33±23.71w-e	88.67±10.93de	82.67±5.90e	113.50±11.39K
Hyderabad	W1	312.00±2.89a-m	348.67±7.88a-h	343.67±11.84a-i	337.00±9.85a-j	335.33±5.66A
	W2	333.00±5.20a-j	355.33±2.91a-f	314.00±14.73a-l	290.33±2.60c-p	323.17±7.99AB
	W3	308.33±18.11a-n	363.33±15.77a-e	365.00±17.39a-d	283.00±4.16f-q	329.92±12.44A
	W4	279.33±13.25f-q	344.33±10.68a-i	321.00±13.86a-l	293.00±3.21c-o	309.42±8.93ABC

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).



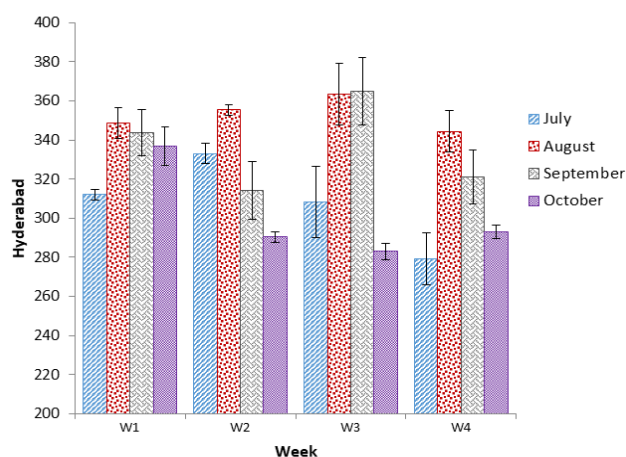


Figure 4. Population of Indian meal moth in Hyderabad during four months

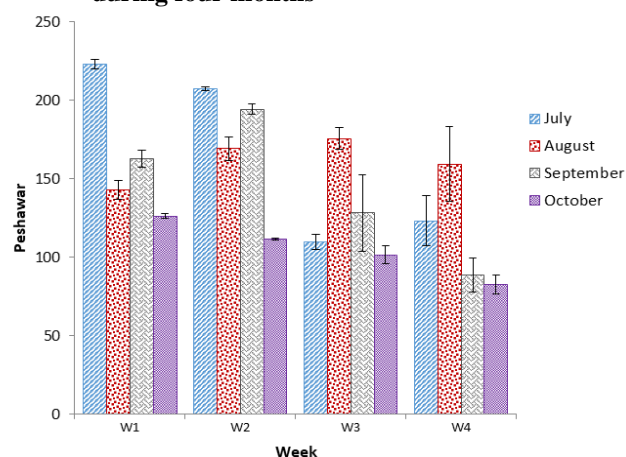


Figure 5. Population of Indian meal moth in Hyderabad during four months

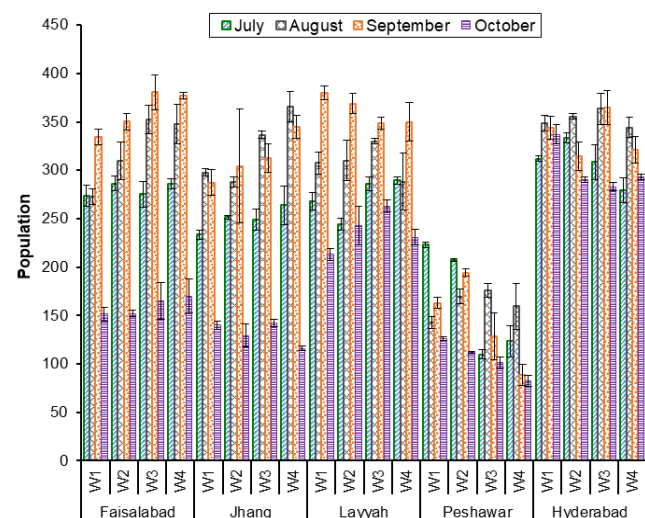


Figure 6. Population of Indian meal moth in all districts during four months

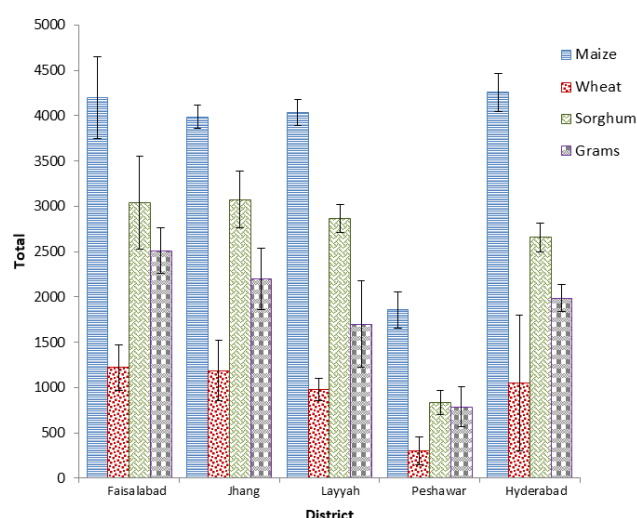


Figure 7. Population of Indian meal moth on different host grains in all districts

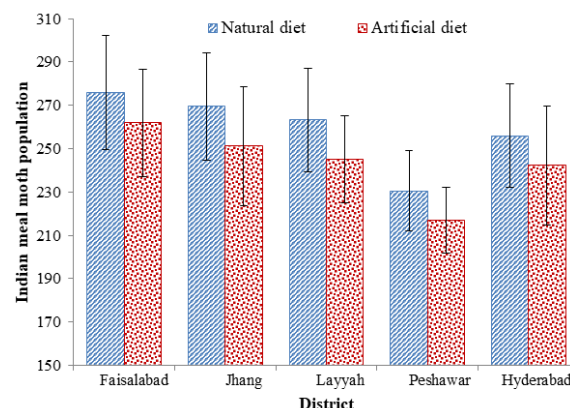


Figure 8. Population of Indian meal moth on natural and artificial diet

Statistical analysis of collected samples: The statistical findings showed that *P. interpunctella* population densities varied for each locality. Districts Faisalabad, Jhang, Layyah, and Hyderabad have greater rates, whereas District Peshawar has lower rates. It's possible that the poor environmental conditions are to blame for the decline in *P. interpunctella* population in Peshawar district.

The statistical analysis showed that *P. interpunctella* population densities on various stored grains, including as wheat, maize, grammes, and sorghum, varied across all districts. The greatest *P. interpunctella* population, however, was found on wheat and maize grains. Different host grains have varied ratios of carbohydrates, fats, and proteins, which may explain the variation in fecundity rates.



DISCUSSION

Identification of *Plodia interpunctella*: Using an electron microscope, all of the *plodia interpunctella* samples that were obtained were identified based on their morphology. The adults had a length of 12.8 mm and a wing span of 16 to 20 mm. The insect's thorax and head were both grey in color.

Rearing of *Plodia interpunctella*: *Plodia interpunctella* was raised in a laboratory under semi-stored grain conditions using a sample population that was taken from five distinct areas in Pakistan. *P. interpunctella* were raised in plastic boxes with grains inside at the study lab. Data on raising was taken every week on the appropriately handled *Plodia interpunctella*.

Conclusion: The study shows that *Wolbachia pipientis* is gram-negative bacteria which symbionts of *Plodia interpunctella*. The destruction of *Plodia interpunctella* is maximum in the city of Faisalabad, and minimum in the city of Hyderabad. The *Plodia interpunctella* was identified by using microscope on the base of morphology.

Conflict of interest: No conflict of interest is declared by authors

Acknowledgement: I would like to acknowledge my all colleagues who helped me to conduct this trail and for their ethical support.

Consent for publication: All authors are giving the consent to publish this research article in JGIAS.

Authors' contributions: Ehsan Bilal Raza, Sajid Abbas Khan and Muhammad Shahid Anees conducted this research, Muhammad kamal and Dr. Samrah Masud maintained this research, Awais Hamza and Muhammad Tahir helped in writing the article, Muhammad Asghar and Gul Zahra Khan proof read the article, Muhammad Shahid Nawaz, helped to revise the article.

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